Center for Veterinary Biologics
and
National Veterinary Services Laboratories
Testing Protocol

Supplemental Assay Method for Sterility Testing of Live Viral Vaccines and Master Seed Virus Samples

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Supplemental Assay Method for Sterility
Testing of Live Viral Vaccines and Master Seed Virus Samples

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1. Introduction

This Supplemental Assay Method describes the test procedure used to detect viable bacteria and fungi in all live viral vaccines and Master Seed Virus samples as prescribed in the Code of Federal Regulations, Title 9 (9 CFR), Part 113.27. In the presence of these contaminating extraneous agents, the medium will be rendered turbid by macroscopic examination.

2. Materials

2.1 Equipment/instrumentation

Equivalent equipment or instrumentation may be substituted for any brand name listed below.

2.1.1 Individually packaged sterile syringes, 1 cc and 3 cc

2.1.2 Vacutainer™ needles

2.1.3 30°-35°C incubator

2.1.4 20°-25°C incubator

2.1.5 Bunsen burner

2.1.6 Biosafety cabinet

2.1.7 Individually packaged sterile pipettes, 1 cc

2.2 Reagents/supplies

Equivalent reagents or supplies may be substituted for any brand name listed below.

2.2.1 Soybean Casein Digest Medium (SCDM) or Trypticase Soy Broth (TSB) **Section 8.1**

2.2.2 Glassware: tubes and flasks with media

2.2.3 Sterile water in serum vials: volumes determined by products to be tested

2.2.4 Sterile clothes: coveralls, mask, hair bonnet, sleeves, shoe covers, and gloves

2.2.5 70% ethyl alcohol

2.2.6 0.05% Germ Warfare disinfectant

2.2.7 Sterile gauze pads, 4 x 4 in

2.2.8 Clean-Pal wipes

3. Preparation for the test

3.1 Personnel qualifications/training

3.1.1 The personnel performing the test must have experience or training in this protocol. This includes knowledge of aseptic biological laboratory techniques and preparation, proper handling, and disposal of biological agents, reagents, tissue culture samples, and chemicals. The personnel must also have knowledge of safe operating procedures and policies and Quality Assurance (QA) guidelines of the Center for Veterinary Biologics-Laboratory (CVB-L) or equivalent, as well as training in the operation of the necessary laboratory equipment listed in **Section 2.1**.

3.2 Preparation of equipment/instrumentation

3.2.1 Turn the biosafety cabinets on at the beginning of the work week and leave them on all week.

3.2.2 Monitor the incubators daily for temperature according to the current version of GDOCSOP0001.

3.2.3 Monitor freezers and coolers used for the storage of biologicals for temperature daily, according to the current version of GDOCSOP0003.

3.3 Preparation of reagents/control procedures

3.3.1 Positive Controls: Use *Bacillus subtilis*, *Candida krusei,* and *Clostridium chauvoei* (or equivalent organisms as specified in the current United States Pharmacopoeia [USP]) as the positive controls in order to determine the growth-promoting qualities of the medium according to 9 CFR 113.25(b). Conduct these positive control tests on each autoclave lot of media according to the current versions of STSAM0900 and STSAM0901.

3.3.2 Prepare the *Bacillus subtilis* and *Candida krusei* reagents according to the current version of the STRPP0001 protocol.

3.3.3 Technique Controls: For each test day, inoculate each of 20 test vessels of media with 0.5 ml of sterile water from serum vials of the same size as used to rehydrate those biologicals tested. Use all volumes of water used with the tested biologicals for the tech controls. If no water vials were needed with the tested biologicals, then inoculate each of 20 test vessels with 0.5 ml of water from 10 serum vials containing 5 ml of sterile water each. Use the same boxes or lots of syringes used with the tested serials of biologicals to inoculate the sterile water of the tech controls. Incubate 10 tech control media test vessels at each incubation temperature for the 14 days of the test.

3.3.4 Negative or Media Controls: Incubate 20 representative test vessels of uninoculated media, per autoclave load, to confirm the sterility of the autoclaved media (9 CFR 113.25(c)). Incubate 10 representative test vessels at each incubation temperature for the 14 days of the test.

3.4 Preparation of the samples

3.4.1 Receive the biological samples to be tested from the Biological Materials Processing Section (BMPS) according to the current version of STSOP0001.

3.4.2 Log in the biological samples by comparing the serial numbers of all vials, recording the diluent numbers, assigning a test number, and completing the testing log book as stated in the current version of STSOP0011.

3.4.3 Determine the volume of test media needed for each serial to be tested on the dilution of preservative computer file using Lotus Approach 97 as described in the current version of STSOP0020. Record volumes used in the log book.

3.4.4 Order sufficient SCDM (Media #10423) from the media preparation department to be delivered 1 day before the biological samples are to be tested. Order sufficient media to include testing for positive, negative, and tech controls.

3.4.5 Order sterile purified water in serum vials from the media preparation department in sufficient volumes, as stated on the label or in the outline, for those serials without accompanying diluent. Order enough sterile water for the tech controls.

4. Performance of the test

4.1 On the day of the test, disinfect the vials of the serials of biologic to be tested with 0.05% Germ Warfare using a Clean-Pal or equivalent. Pay special attention to the cleaning of the tops of the vials and rubber stoppers.

4.2 Set the disinfected vials on a tray and place the tray on a table inside the outer sterility room.

4.3 Gown up for doing the sterility test by wearing sterile coveralls, booties, sleeves, mask, hair bonnet, safety glasses, and gloves.

4.4 Disinfect the interior surfaces of the biosafety cabinet used for testing with 70% alcohol or equivalent.

4.5 Number the media test vessels to coincide with the serials to be tested.

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4.6 Place the testing materials (syringes, Vacutainer needles, 4 x 4-in gauze squares, etc.) in the biosafety cabinet or on a cart next to the cabinet.

4.7 Place the samples and test media for the first serial in the biosafety cabinet.

4.8 Disinfect the tops of the samples with a 4 x 4-in gauze pad soaked in 70% alcohol. Then flame the tops of the samples using a Bunsen burner. Equivalent methods for decontaminating the tops of the samples may be used.

4.9 Dehydrate each vial of the serial, if desiccated, using a syringe and needle or Vacutainer needle. Use the firm's diluent if provided, and sterile water if not. Rehydrate products used for mass inoculation by water or spray, with no accompanying diluent, with sterile water at a rate of 30 ml per 1000 doses or the amount of diluent specified on the vial. Consecutively rehydrate 10 vials of each serial. When testing Master Seed Virus samples and 10 vials are not available, at least 4.5 ml of Master Seed Virus is needed for testing.

4.10 Inoculate 0.2 ml from each vial of the dehydrated liquid or thawed frozen liquid product into each of 2 test vessels of media. Determine the volume of media needed in these test vessels by the method described in **Section 3.4.3**. If less than 10 vials of Master Seed Virus are received, divide the Master Seed inoculum among the 20 test vessels, 0.2 ml per test vessel.

4.11 Repeat **Sections 4.7-4.10** on the other serials of biologic to be tested this day.

4.12 Prepare the tech controls by inoculating 20 test vessels with approximately 0.5 ml of sterile water, using syringes and needles of the same lot used with the tested serials.

4.13 Place the test vessels in the appropriate incubator temperature depending on the volumes of media indicated in **Section 3.4.3** for each incubator temperature. Also place 10 tech control test vessels at each incubator temperature and 10 negative or broth control test vessels at each

incubator temperature. After putting the serials on test, initial and date the log book for this test code.

4.14 Clean the sterility room by disinfecting the interior of the biosafety hood and counter tops with 70% alcohol. Remove paper trash from the sterility room and discard the biological samples and any extra media by autoclaving.

5. Interpretation of the test results

5.1 On day 14 of the incubation period, examine all test vessels for cloudiness due either to the product or a contaminant. If it is not possible to determine if the cloudiness is due to a contaminant, then subculture the serial. To subculture, place 1 ml from the test vessel into 40 ml of fresh SCDM, using a sterile individually packaged 1-ml pipette. If less than 10 test vessels of a serial are cloudy, then subculture those that are or at least 3 vessels. If all 10 test vessels are cloudy, then subculture 3 randomly picked test vessels at each incubation temperature. Incubate these subculture tubes for an additional 3 days.

5.2 Prepare 1 microscope slide from each tube which appears to have macroscopic growth. After these slides have dried, Gram stain and observe them with a microscope. Enter the number of tubes with growth and no-growth, as well as the Gram stain results, in the log book for this test code. Initial and date the log book as the person taking the serials off test.

5.3 If extraneous growth is observed in 2 or 3 test vessels and confirmed by Gram stain, then conduct 1 retest using 20 unopened final container samples.

5.4 If no extraneous growth is found in 9 or 10 test vessels of the initial test or 19 or 20 vessels of the retest, the serial is satisfactory (SAT).

5.5 If extraneous growth is found in 4 or more test vessels of the initial test or 2 or more of the retest, the serial of biologic is unsatisfactory (UNS).

5.6 If a serial is found unsatisfactory, freeze 3-4 ml of contaminated media in the Cytology/Sterility (CY/ST) section's central Revco. Label the tube containing the 3-4 ml with the test code, the serial's test number, and the date. Save the Gram-stained microscope slides.

6. Report of test results

6.1 Record test results in the testing log book and on the computer test sheet for each serial tested by indicating the number of vessels with no growth over the number of vessels on test and the test conclusion of SAT or UNS. Initial and date the log book and computer test sheet after entering the test results on the 14th day.

6.2 Enter the results recorded on the test sheet in the computer. A computer printout of the result will be generated. Compare these printouts against the test sheet and log book for accuracy. Review the current version of STSOP0021 for directions on entering test results.

6.3 Forward the test result printouts to the CY/ST microbiologist or supervisor to review, sign, and date.

6.4 Validate the test results in the computer, according to the current version of STSOP0021.

6.5 File the signed and validated test report printouts in the CY/ST files under the first 2 numbers of each serial's product code. File the BMPS test sheet, by test code, in the same file drawer.

7. References

7.1 Code of Federal Regulations, Title 9, Parts 113.25 & 113.27, U.S. Government Printing Office, Washington, DC, 1998.

7.2 The U.S. Pharmacopoeia, 1985, Vol. 21, pp 1151-1160, Mack Publishing Co., Easton, PA.

8. Appendix

8.1 NVSL Media Formulation # 10423

SOYBEAN CASEIN DIGEST MEDIUM (SCDM)
Or
TRYPTICASE SOY BROTH (TSB)

Trypticase Soy Broth	30 g
QH_2O	1000 ml

Autoclave 20 minutes at 121°C.

TSB and SCDM are 2 names for the same media formulation from different media companies.

9. Changes

The information contained in this document was previously available as a protocol (STPRO0270.01 dated September 9, 1996). The document was rewritten to meet the current NVSL/CVB QA requirements, to clarify practices currently in use in the CVB-L, and to provide additional detail. No significant changes were made from the previous protocol.